FORMATION OF THE BOUNDARY BETWEEN CENTRAL AND PERIPHERAL NERVOUS SYSTEM

Mamiti Yoshida, Hiroshi Nakayasu
Department of Biology, Okayama University, Okayama 700-8530, Japan

There is clear boundary between the central and peripheral nervous system. It is not yet known how the boundary formed. The peripheral part of axons, like motor neurons and sensory neurons, are covered by Schwann cells. On the contrary, the central part of these axons are covered by oligodendrocytes. Schwann cells will come into collision with the oligodendrocytes at the some point on the axon, and this point could be the boundary. This idea is quite likely, but our results antagonized the possibility. Monoclonal antibody (YM-8) stained the peripheral part of the axons of cranial and spinal neurons in zebrafish brain. Usually, Schwann cells appear at six weeks after fertilization, but the YM-8 staining appeared at the 2 weeks after the fertilization. Furthermore, the antibody did not stain Schwann cells. These results indicate that the YM-8 antigen is located on the axons before the covering by Schwann cells.

INVOLVEMENT OF NITRIC OXIDE IN ODOR DISCRIMINATION IN THE LAND MOLLUSK *LIMAX VALENTIANUS*

Satoshi Watanabe, Midori Sakura, Yutaka Kirino
Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan

The procerebrum (PC) of the land slug *Limax valentianus* is the second order olfactory center and contains about 100,000 neurons. The membrane potentials of these neurons oscillate regularly, and a regular local field potential oscillation is recorded from the surface of the PC. The oscillatory activity is nearly synchronous throughout the PC, but it has a phase gradient in such a way that the activity of the apical neurons is advanced relative to the basal neurons. Odor stimulus to the tentacle epithelium or electrical stimulation of the tentacle nerve modulates the frequency and synchronicity of the oscillation. We found that L-NAME, an inhibitor of nitric oxide synthesis, blocks the changes in the oscillation frequency and synchronicity induced by stimulation of the tentacle nerve. We also made behavioral analysis of odor discrimination using aversively conditioned slugs, and found that L-NAME injected into the body cavity 1 hr prior to behavioral tests blocks discrimination between similar odors. These results suggest that nitric oxide-mediated modulation of the PC activity plays an important role in odor discrimination.

NEURONS RELATED TO OSMOTIC CONTROL IN ZEBRAFISH BRAIN

Yuuko Nakabayashi, Hiroshi Nakayasu
Department of Biology, Okayama University, Okayama 700-8530, Japan

We produced a monoclonal antibody (YN-1) which specifically recognizes an unknown large neuron in zebrafish brain. About twenty neurons are found at the level of third cranial nerve nucleus. The neurons seems to extend their axons to prolactin cells in pituitary gland. There are another YN-1 positive neurons at the caudal end of medulla. These neurons seems to extend to the salt cells in the gill and also to the renal tubes in the mesonephros. These results suggested that the YN-1 positive neurons could be related to the osmotic control in the zebrafish body.

TOWARD THE VISUALIZATION OF TARGETED NEURONS USING TRANSGENIC SILKWORM, *BOMBYX MORI*

Tomoko Yamagata[1], Ryohei Kanzaki[2], Keirou Uchino[3], Toshio Kanda[3], Toshiki Tamura[3]
[1]College of Biological Sciences, Second Cluster of Colleges, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8572, Japan, [2]Department of Mechano-Informatics, Graduate School of Information Science and Technology, University of Tokyo, Bunkyo-ku, Tokyo 113-8656, Japan and [3]Insect Gene Engineering Laboratory, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan

Insect brains have great advantage to study the neural network since their brains consist of relatively small number of neurons. Especially in the brain of silkworm, Bombyx mori, many studies had done morphologically, physiologically, and by neurobehavioral approach. As for it, the visualization of the innervating of single neurons without damaging them will rapidly progress the study of nervous system. Silkworm also has strong genetic tools such as transgenic system and detailed genetic maps. In this study, we tried to apply the genetic engineering techniques of the silkworm for the study of the insect nervous system. Previous studies showed that the GAL4/UAS gene regulation system works in silkworm. Applying this system, we examined whether the animal will express GFP in targeted neurons in the brain. As a result, subsets of the targeted neurons are successfully visualized by specific GAL4 drivers, and it appears that the GFP expression patterns of individual animals are quite consistent among each GAL4 drivers.

SEROTONIN CHANGES THRESHOLD OF THE NEURAL RESPONSE IN THE PRIMARY CENTER OF THE MALE MOTH OLFACTORY SYSTEM

Mitsuhiro Saito[1], Koutaroh Okada[2], Ryohei Kanzaki[2]
[1]Institute of Biological Sciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba-shi Ibaraki 305-8572, Japan and [2]Department of Mechano-Informatics, Graduate School of Information Science and Technology, the University of Tokyo, Bunkyo-ku Tokyo 113-8656, Japan

It is known that serotonin at 10^{-4}mol l^{-1} raises the behavior sensitivity of the male silkmoth *Bombyx mori* to pheromone and causes reversible increases in the optical responses in the macroglomerular complex (MGC) of the male *B. mori* primary center of olfactory system, the antennal lobe (AL), by electrical stimulation of the antennal nerve (AN). Here, in order to examine the role of serotonin on the threshold of neuronal response, we utilized high-speed optical imaging with a voltage-sensitive dye RH414 combined with bath application of serotonin. We used isolated *B. mori* brains and evoked neuronal response with electrical stimulation, using amplitudes ranging from 10μA to 100μA at steps of 10μA interval to the AN. Furthermore we analyzed the spatio-temporal change of the neuronal response in MGC of male *B. mori* AL. Consequently, we observed that serotonin decreases the threshold of the neuronal response in the MGC, which consists of neurons responding specifically to pheromone. Our results show that serotonin changes the threshold of neuronal responses to pheromone, which may be related to the serotonin modulation of the pheromone sensitivity at the behavioral level.

CREB ISOFORMS IN CENTRAL NERVOUS SYSTEM OF POND SNAIL *LYMNAEA STAGNALIS*

Hisayo Sadamoto[1], Akiko Wagatsuma[2], Kenta Saito[3], Masataka Kinjo[3], Etsuro Ito[1,2]
[1]Division of Innovative Research, Creative Research Initiative "Sousei", Hokkaido University, North 21, West 10, Kita-ku, Sapporo 001-0021, Japan, [2]Division of Biological Sciences, Graduate School of Science, Hokkaido University, North 10, West 6, Kita-ku, Sapporo 060-0810, Japan and [3]Section of Intelligent Materials and Device, Research Institute for Electronic Science, Hokkaido University, North 12, West 6, Kita-Ku, Sappro 060-0812, JAPAN

Cyclic AMP-responsive element binding protein (CREB) has multiple functions in gene regulation. We characterized seven CREB1 mRNA isoforms of spliced variants in the central nervous system (CNS) of the pond snail *Lymnaea stagnalis*. The three isoforms, coding a whole CREB1 protein, are considered to be the activators for gene regulation. The other four isoforms, coding the truncated CREB1 proteins without a kinase inducible domain, are the repressors. The expression level of these isoforms in *Lymnaea* CNS was investigated by a real-time qRT-PCR method. As well as the activator isoforms, the repressor isoforms were abundantly expressed, and the expression of all isoforms was changed by stimulation of high concentration of potassium chloride. These findings showed that both isoforms are expressed at large amount in the CNS, suggesting that different CREB isoforms contribute gene regulation in the CNS by changing the ratio between different isoforms. Furthermore, we have induced the expression of these isoforms fused to fluorescent protein (GFP and RRFP) in HeLa cells, in order to visualize the intracellular colocalization.

ANALYSIS OF A NOVEL HONEYBEE BRAIN SPECIFIC CELL ADHESION MOLECULE (*AbsCAM*)

Masahiro Funada[1], Hiroaki Hara[2], Hiromi Sasagawa[3], Yasuo Kitagawa[1], Tatsuhiko Kadowaki[1]
[1]Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan, [2]School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan and [3]Life Science and Bioengineering, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

We isolated a novel honeybee gene *AbsCAM*, which encodes cell adhesion molecule consisting of nine Ig domain, six FNIII domain, transmembrane domain, and intracellular domain. *AbsCAM* is specifically expressed in honeybee brain, and the level of expression decreases by age in worker bees. We now characterize the function of *AbsCAM* for pathfinding of neurons in the central and peripheral nerve systems of *Drosophila* by the ectopic expression. Furthermore, we are in the middle of establishing the knock-down of *AbsCAM* in honeybee brain by RNA interference technique.

ANALYSIS OF A NOVEL GENE, *MAHYA* IDENTIFIED FROM HYMENOPTERA

Mayumi Tsuchimoto[1], Shinobu Yasuo[1], Takashi Yoshimura[1], Hiromi Sasagawa[2], Osamu Tadauchi[3], Tatsuhiko Kadowaki[1]
[1]Graduate School of Bioagricultural Sciences, Nagoya University, Cikusa, Nagoya 464-8601, Japan, [2]Foundation for Advancement of International Science, Tsukuba, Ibaraki 305-0062, Japan and [3]Graduate School of Bioresource and Bioenvironmental Sciences Hakozaki 6-10-1, Higashi-ku, Fukuoka 812-8581, Japan

We identified a gene, *Mahya* which encodes a novel secretory protein from the eusocial insect, honeybee(*Apis mellifera*). Although the genes homologous to *Mahya* exist in the vertebrate, it is not present in two Dipteran species (*Drosophila melanogaster* and *Anopheles gambie*), nematode, and ascidian. Meanwhile, we have identified two *Mahya* genes (*Mahya-1* and *Mahya-2*) in zebrafish, mouse, and human. In mouse brain, *mMahya-1* is expressed in the olfactory bulb, cerebellum, and hippocampal CA3 region, and the expression level of *mMahya-2* is low but detected in the hippocampal CA3 region and the dentate gyrus granular cell layer.